Title: Single MHC-I expression promotes virus-induced liver immunopathology

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Abstract

MHC-I molecules present epitopes on the cellular surface of antigen-presenting cells to prime cytotoxic $CD8^+$ T-cells (CTLs) which than identify and eliminate other cells such as virus-infected cells bearing the antigen. Human hepatitis virus cohort studies have previously identified MHC-I molecules as promising predictors of viral clearance. However, the underlying functional significance of these predictions is not fully understood. Here, we show that expression of single MHC-I isomers promote virus-induced liver immunopathology. Specifically, using the lymphocytic choriomeningitis virus (LCMV) model system, we found MHC-I proteins to be highly upregulated during infection. Deletion of one of the two MHC-I isomers H2-Db or H2-Kb in C57Bl/6 mice resulted in CTL activation recognizing the remaining MHC-I with LCMV epitopes in increased paucity. This increased CTL response resulted in hepatocyte death, increased caspase activation and severe metabolic changes in liver tissue following infection with LCMV. Moreover, depletion of $CD8^+$ T cells abolished LCMV induced pathology in these mice with resulting viral persistence. In turn, NK cell depletion further increased anti-viral CTL immunity and clearance of LCMV even in presence of a single MHC-I isomer. In conclusion, our results suggest that uniform MHC-I molecule expression promotes enhanced CTL immunity during viral infection and contributes to increased CLT mediated liver cell damage that was alleviated by CD8 or NK cell depletion.

Introduction

The liver is a vital organ with a variety of functions in metabolism including carbohydrate, lipid, and nitrogen metabolism(1, 2). Moreover, the liver is involved in plasma protein expression and detoxifications(3). Furthermore, venous blood from the gut directly feeds into the liver via the portal vein, potentially exposing the liver to a great variety of pathogens(4). Hepatotropic pathogens such as hepatitis B virus (HBV) or HCV can establish chronic infections and causing the life-threatening liver disease to hundreds of millions of people worldwide(1, 5, 6). The liver composes multiple cell types including hepatocytes (HC), hepatic stellate cells (HSCs), Kupffer cells (KCs), and liver sinusoidal endothelial cells (LSECs)(7). Under inflammatory conditions, myeloid cell-derived inflammatory monocytes named iMATEs can be also found in liver tissues to promote anti-pathogen immunity(8).

Cytotoxic T cell (CTL) immunity contributes to the clearance of viral infections by eliminating infected cells. Major histocompatibility complex (MHC)-I molecules along with their cofactor beta-2-microglobulin (β2m) present peptides on the cell surface of nucleated cells to make them recognizable to CTLs. All hepatic cell types express MHC-I with varying functions(7). While antigen presentation via MHC-I on iMATEs leads to enhanced T cell proliferation(8), hepatocyte-derived MHC-I presentation may contribute to deletion of CTLs(9). The contact of CTL and LSECs via MHC-I can determine the formation of long-lived memory $CD8^+$ T cells(10). Moreover, although increased MHC-I expression can increase T cell activation, prolonged viral infection with the presentation of high antigen levels on MHC-I molecules can contribute to exhaustion of anti-viral T cells and consequently cause the establishment of chronic viral infection(11, 12).

Three maternal and Three paternal inherited MHC-I isomers define an individual's set of antigen-presenting molecules called human leukocyte antigen (HLA) class in

humans and histocompatibility antigen 2 (H2) in mice(13). An increased repertoire of peptide presentation by heterozygous expression of HLA molecules can trigger a broader T cell response preventing viral escape and accordingly benefit viral clearance and is therefore referred to as the HLA heterozygosity advantage. Previous studies have highlighted the importance of HLA in human chronic infectious diseases. Specifically, heterozygous expression of HLA class II molecules showed better viral clearance than homozygoes expression in patients suffering from HBV or HCV infection (14-16). Consistently, disease progression during HIV infection is inversely correlated with heterozygous HLA class I expression(17). However, during infection with hepatitis viruses the role of heterozygous HLA class I expression is less clear. Only a minor correlation between HLA class I heterozygosity and HCV viral clearance was observed(16). Furthermore, progression to liver fibrosis was not associated with heterozygous or homozygous expression of HLA –A, -B, or –C(18). However, the expression of certain HLA class I genes can influence the outcome of infections with hepatitis viruses. Analysis of HLA class I alleles during HCV infection between patients who cleared infection or who developed chronic infections revealed that the alleles B*15, B*27, B*57, and C*01 were associated with effective HCV clearance. In contrast, expression of HLA-A*23 was associated with viral persistence of HCV(19-23). Taken together, while there is evidence that MHC-I molecule expression influences the course of viral infection in the liver, the role of their expression on the course of infection and pathology during viral infections are insufficiently understood. The inbred, genetically homozygous mouse strain C57BL/6 expresses only the two MHC-I molecules H2-Db and H2-Kb(24). In mice, infection with the noncytopathic lymphocytic choriomeningitis virus (LCMV) can also affect hepatocytes, which results in anti-viral CTL-dependent hepatitis(25). Accordingly, LCMV can be used as a model to investigate CTL-mediated liver immunopathogenesis(26). Notably, H2-Db and H2-Kb share the similar

106 immunodominant epitope gp33 and gp34 of LCMV respectively (27), which reduces the
107 probability of viral escape in experimental model systems where specific MHC-I
108 molecules are deleted.

109 In this study, we show that MHC-I expression is highly upregulated following
110 chronic LCMV infection in liver tissue. Moreover, the expression of uniform MHC-I
111 molecules in H2-Db and H2-Kb deficient mice resulted in severe liver immunopathology.
112 While T cell immunity was only restricted to the remaining MHC-I isomer, increased T
113 cell numbers were detected. The liver pathology was associated with increased liver cell
114 death and slightly accelerated clearance of LCMV indicating an increased CTL immunity.
115 Depletion of $CD8^+$ T cells abolished LCMV mediated immune pathology while NK cell
116 depletion boosted the uniform CTL response to clear LCMV in absence of severe
117 pathology.

Materials and Methods:

Mice, Viruses, Virus Titration, and Cell Depletion:

H2-D1$^{-/-}$, *H2-K1*$^{-/-}$ mice were bred in a C57BL/6 background and maintained under specific pathogen-free conditions. Experiments were performed under the authorization of LANUV in accordance with German laws for animal protection. LCMV strain Docile was originally obtained from Dr. C. J. Pfau (Troy, New York). Viruses were propagated in L929 cells (obtained from ATCC, NCTC clone 929) and virus titers were measured using a plaque-forming assay as previously described (28). Briefly, organs were harvested into HBSS and homogenized with a tissuelyser (Qiagen). Diluted virus samples were mixed with MC57 cells in 24 well plates. After 3hours, methylcellulose 1% medium was added. After 2 days of incubation, viruses were visualized by staining against LCMV nucleoprotein via an anti-LCNV-NP antibody (clone: VL-4). $CD8^+$ T cells were depleted with intravenous (i.v.) injection of anti-CD8 antibody (clone YTS169.4)(28), $NK1.1^+$ cells (clone PK136) were depleted with intravenous (i.v.) injection of anti-NK1.1 antibody(29).

Histology: Histological analysis of snap-frozen tissue was performed as previously described(28). Antibodies against cleaved Caspase-3, cleaved Caspase-8 (Cell Signaling) and self-made anti-LCMV monoclonal antibody (Clone: VL4), were used. TUNEL staining was performed using the *In Situ* cell death detection kit, fluorescein (Roche) as per manufacturers' instructions. Images were acquired with the ZEISS LSM 880 or ZEISS Axio Observer Z1 microscopes. Cleaved Caspase-3, cleaved Caspase-8, and TUNEL quantifications were analysed by ImageJ software.

Flow cytometric analysis: Experiments were performed using a FACS Fortessa and analyzed using FlowJo software. For cell subsets and surface molecules staining, single suspended cells were incubated with antibodies (anti-CD19, CD3, CD4, CD8, F4/80,

CD202b, HNF4a, H2Db, H2Kb (Thermo Fisher Scientific)) for 30 min at 4°C. Tetramer and intracellular cytokine staining were performed as described previously (29). For tetramer staining, single suspended cells were incubated with tetramer-gp33 or tetramer-gp34 (CD8) for 15 minutes at 37°C. After incubation, surface antibodies (anti-CD8, IL-7R, KLRG1, CD44, CD62L, PD-1, 2B4, TIM-3, LAG-3, CXCR5) were added for 30 minutes at 4°C. For intracellular cytokine re-stimulation, single suspended cells were stimulated with LCMV specific peptides gp33, gp34 (27) for 1 hour. Brefeldin A (Thermo Fisher Scientific) was added for another 5 hour incubation at 37°C followed by staining with anti-CD8 antibody (Thermo Fisher Scientific). After surface staining, cells were fixed with 2% formalin, followed by permeabilization with 0.1% Saponin, and stained with anti-IFN-γ for 30 min at 4°C.

RNA purification and RT-PCR: RNA was isolated using Trizol according to the manufacuror's instructions (Thermo Fisher Scientific). RT-PCR was performed using the iTaq Universal SYBR GreenOne-Step RT-qPCR Kit (Biorad) as previously described (REF). For analysis, expression levels were normalized to *Gapdh*.

Immunoblotting: Liver tissue was smashed using a tissue lyser (TissueLyser II, QIAGEN) and lysed using SDS lysis buffer (1.1% SDS, 11% glycerol, 0.1 mol/L Tris, pH 6.8) with 10% β-mercaptoethanol. Blots were probed with anti-α-tubulin (Merck), anti-Caspase-3, anti-Caspase-8, or anti-Beta-2 microglobulin (Cell Signaling) followed by dection with the Odyssey infrared imaging system (Odyssey Fc, LI-COR Biosciences). Immunoblots were quantified using ImageJ.

Metabolomics: Targeted metabolomics profiling of liver samples was performed using the AbsoluteIDQ p180 Kit (Biocrates LifeSciences AG). This kit allows for absolute

quantification of 188 metabolites. The measurements were carried on a Xevo TQ-S tandem mass spectrometer coupled to an Acquity UPLC-I class system (ultra-performance liquid chromatography). Blood glucose levels were measured using a Bayer Contour Blood Glucose Meter.

Statistical analysis: Data are expressed as mean ± S.E.M. For analysis of statistical significance between two groups, a Student t-test was used. For analysis of multiple time point experiments, two-way ANOVA with an additional Bonferroni post-test was used. *$p<0.05$, **$p<0.01$, ***$p<0.001$ was considered statistically significant. Survival curves were analyzed by Log-Rank (Mantel-Cox) test.

Results

Uniform MHC-I expression results in liver tissue damage following infection with LCMV.

Liver cell apoptosis can be induced by CTLs during non-cytolytic LCMV infection(30). To study the role of heterozygous MHC expression following CTL-induced liver damage during infection, we infected H2-Db deficient mice on a C57Bl/6 background with the LCMV. In this model system, *H2-D1*$^{-/-}$ mice will only express H2-Kb as MHC-I, while C57Bl/6 mice will express H2-Db and H2-Kb. We observed a decrease in glucose levels in both WT and H2-Db deficient animals after infection. However, glucose levels were restored in control animals but not H2-Db deficient mice 9 days post-infection, suggesting an impairment of liver function in absence of H2-Db (Figure 1a). Notably, 8-9 days post infection, we observed that *H2-D1*$^{-/-}$ mice exhibited hypothermia, hypoactivity, and had a noticeably sullied coat following what is usually an asymptomatic LCMV infection in WT animals (Figure 1b). *H2-K1*$^{-/-}$ animals, which only exhibit uniform expression of H2-Db also appeared in a moribund state, but to a lesser extent than mice expressing H2-Kb (Figure 1b). Damaged liver tissue can be detected by an increase of hepatic enzymes in the bloodstream. Elevated liver enzymes can be observed during LCMV infection as a result of $CD8^+$ T cell-mediated immunopathology(25). We found increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) enzyme activities in the serum of LCMV infected H2-Db deficient mice compared to WT mice (Figure 1c). Serum ALT levels were under the detection limit in naïve WT, H2-Db deficient or H2-Kb deficient mice (Figure S1a). Liver tissue damage may lead to liver metabolic dysfunction(30). Consistently, we observed that glycerophospholipids were highly increased in WT infected liver tissue when compared to H2-Db deficient liver tissue (Figure S1b). In turn,

long-chain acyl-carnitines were highly increased in the liver tissue of LCMV infected H2-Db deficient mice when compared to WT counterparts (Figure S1c). Additionally, we found most of the amino acids were down-regulated in liver tissues when H2-Db was missing (Figure S1d). These data suggest a decreased metabolic function following infection in presence of uniform MHC-I expression. Biogenic amines showed a mixed expression pattern: 4-Hydroxyproline, methionine sulfoxide, and asymmetric dimethylarginine expression were decreased whereas kynurenine, serotonin, and aminoadipic acid expression were increased in H2-Db deficient liver tissue (Figure S1e). High kynurenine expression might be associated with increased hepatic inflammation during LCMV infection(31). However, serotonin can affect T-cell immunity and is associated with increased liver pathology(28). Taken together, these data suggest that uniform MHC-I expression results in severe liver damage, hepatic metabolic dysfunction, and severe pathology following infection with LCMV.

MHC-I expression is up-regulated after LCMV infection in liver tissue.

Since H2-Db deficient animals exhibited severe liver pathology following LCMV infection, we wondered whether hepatic MHC-I protein expression changed after infection. Previous findings have shown that MHC-I can be up-regulated in the central nervous system during LCMV infection (32). Consistently, liver *H2-D1* and *H2-K1* mRNA expression increased after LCMV infection (Figure 2a). Furthermore, we observed that the Beta-2 microglobulin expression levels were significatlly increased in liver tissue following infection (Figure 2b). Using flow cytometry, H2-Db and H2-Kb expression was observed on F4/80^{+} (Kupffer cells), Tie2^{+} endothelial cells, and HNF4a^{+} hepatocytes following infection in the liver tissue (Figure 3b, Figure S2a+b). These data indicate that MHC-I expression is increased during LCMV induced liver inflammation, and this increase is observed on multiple hepatic cell types. H2-Db and H2-Kb expression was also

confirmed on several immune cell subsets in single-cell suspended splenocytes, including $CD19^+$ B cells, $CD4^+$ T cells, $CD8^+$ T cells, and $F4/80^+$ macrophages following infection (Figure 2d, Figure S2c+d). Taken together, these data indicate that MHC-I molecules are highly and ubiquitously upregulated following infection on multiple cells including immune subsets in liver and spleen tissue.

H2-Db expression prevents liver cell apoptosis during LCMV infection.

MHC-I molecules can recruit anti-viral $CD8^+$ T cells, which mediate liver cell death during LCMV infection(25). Specifically, liver sinusoidal endothelial cells can contribute to $CD8^+$ T cell stimulation and CTL-mediated TNF production(33). Moreover, $CD8^+$ T cells can crawl along liver sinusoids in scanning hepatocellular antigen to induce caspase activation and liver cell death(34). Accordingly, we hypothesized that during uniform MHC-I expression liver cell death was increased. It has been reported $CD8^+$ T cells can trigger liver lesion (hepatic steatosis) during LCMV infection(35). Similarly, liver histology revealed that LCMV infected H2-Db deficient mice had more lesions in liver tissue when compared to liver sections from LCMV infected WT animals (Figure 3a). Notably, we did not observe any significant difference in LCMV infected liver cells or LCMV titers between *H2-D1*$^{-/-}$ and WT animals in this setting (Figure S3a+b). Next, we checked whether the absence of H2-Db triggered increased apoptosis in liver tissue following infection with LCMV. In line with the elevated liver enzymes in *H2-D1*$^{-/-}$ mice, we also observed increased staining of DNA fragmentation by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) in liver tissue of H2-Db deficient mice when compared to WT controls (Figure 3b+c, Figure S4a+b). DNA fragmentation can result from the activation of effector caspases such as caspase 3(36). As expected, we found increased Caspase 3 activation in snap-frozen liver sections of H2-

Db deficient mice when compared to WT controls following LCMV infection (Figure 3d+e, Figure S4c-f). Notably, no Caspase-3 cleavage differences were observed in WT, *H2-D1*$^{-/-}$ and *H2-K1*$^{-/-}$ liver tissue under naïve condtions, suggesting H2-Db or H2-Kb deficiency does not directly cause liver cell apoptosis (Figure S4e+f). Effector caspases are activated by initiator caspases such as caspase-8 (Casp8) during TNFR1 mediated cell death(37). Consistently, active Caspase-8 was also highly expressed in the absence of H2-Db when compared to control animals (Figure 3f+g, Figure S4g-j). These data confirm that the absence of H2-Db resulted in increased liver cell death following LCMV infection.

Effective CTL immunity promotes immunopathology in *H2-D1*$^{-/-}$ mice following LCMV infection.

Hepatocyte death is induced by CTL during LCMV infection. Therefore, we invegated the CTL response in H2-Db deficient mice after LCMV infection. Indeed, we observed an increased frequency of KLRG1^{+}CD62L^{-}IL-7R^{-} effector LCMV-gp34^{+}tet^{+}CD8^{+} (H2-Kb restricted) T cells in the peripheral blood of *H2-D1*$^{-/-}$ mice compared to WT animals (Figure 4a, Figure S5a). KLRG1^{-} CD62L^{+}IL-7R^{+} memory T cell frequency was also increased in *H2-D1*$^{-/-}$ mice (Figure 4a, Figure S5a). A lower dose of LCMV also resulted in increased LCMV-gp34^{+}tet^{+}CD8^{+} T cells in *H2-D1*$^{-/-}$ mice (Figure 4b). KLRG1^{+}CD62L^{-}IL-7R^{-} effector T cells were also increased in this setting (Figure 4c, Figure S5b). We did not observe major differences in expression of exhaustion molecules including PD-1, LAG-3, TIM-3, and 2B4 between WT and *H2-D1*$^{-/-}$ LCMV specific T cells (Figure 4d, Figure S5c). However, when we re-stimulated splenocytes *ex vivo* with a variety of LCMV specific peptides, we detected increased IFN-γ production in CD8^{+} T cells harvested from H2-Db deficient mice compared to WT controls (Figure 4e).

Consistently, LCMV infection also resulted in increased LCMV-gp33^{+}tet^{+}CD8^{+} (H2-Db restricted) and LCMV-np396^{+}tet^{+}CD8^{+} T (H2-Db restricted) cell frequencies in *H2-K1*$^{-/-}$ animals (Figure S5d). We also observed increased KLRG1^{+}CD62L^{-}IL-7R^{-} effector T cells in *H2-K1*$^{-/-}$ mice compared to WT controls (Figure S5e). Consistent with increased effector T cell function, we observed accelerated LCMV control in H2-Db deficient animals in this setting, although both WT and H2-Db deficient animals eventually eliminated LCMV (Figure 4f, Figure S5f). These data indicate that uniform MHC-I expression results in increased CTL immunity against the MHC-I isomer restricted peptides.

Considering the hyper CTL response in H2-Db knockout mice after LCMV infection, we investigated whether depletion of CLT in H2-Db deficient mice can revert the immunopathology. Depletion of CLTs using a CD8^{+} T cell depleting antibody, reduced active Caspase-8, cleaved Caspase-3, and TUNEL levels in snap-frozen liver tissue following infection (Figure 5a-f). Moreover, lesions in liver tissues were not observed in CD8^{+} T cell-depleted *H2-D1*$^{-/-}$ mice (Figure 5g). ALT, AST, and LDH activity following LCMV infection were reduced in the absence of CD8^{+} T cells in *H2-D1*$^{-/-}$ mice, despite high LCMV titer (Figure 5h, Figure S6). These data indicate that CTL immunity following uniform MHC-I expression caused severe hepatic immunopathology.

Overproduction of IFN-γ and/or TNF-α is associated with inflammation, tissue damage, and death in human or animal infection models (38, 39). We observed increased levels of serum IFN-γ but not TNF-α in H2-Db knockout animals (Figure 6a, Figure S7). When mice were treated with an anti-CD8 depletion antibody, IFN-γ was reduced in both WT and H2-Db knockout mice at day 4 post infection (Figure 6a). This finding suggests that CD8^{+} T cells in H2-Db mice were the source of IFN-γ and likely activated to a greater extent than their WT counterparts during the early phase of viral infection. Furthermore,

ablation of CLTs restored glucose levels in both WT and *H2-D1*$^{-/-}$ mice (Figure 6b). Furthermore, survival and liver immunopathology observed in H2-Db deficient mice following LCMV infection were rescued by depletion of CD8^{+} T cells (Figure 6c). Taken together, these data suggest that uniform MHC-I can trigger increased T cell activation and resulting in severe immunopathology. Consistently, CD8^{+} T cell depletion could block immunopathology but promoted LCMV persistence. Next, we wondered whether CD8^{+} T cell immunity could be further boosted to eliminate LCMV and alleviate immunopathology during uniform MHC-I expression. Specifically, NK cells are known to suppress CD8^{+} T cell immunity during LCMV infection. Accordingly, depletion of NK cells leads to faster viral clearance due to enhanced CD8^{+} T cell responses and reduced immunopathology (40-42). In our model system, NK cell-depleted *H2-D1*$^{-/-}$ mice showed increased CD8^{+} T cell immunity than NK cell competent *H2-D1*$^{-/-}$ mice (Figure 6d). As a consequence, LCMV was eliminated in all tissues harvested from NK cell-depleted *H2-D1*$^{-/-}$ mice, but also WT animals (Figure S8). However, NK cell depletion prevented severe immunopathology following LCMV infection when compared to NK cell competent H2-Db deficient mice (Figure 6e). These data suggest that viral replication in liver cells targeted by CD8^{+} T cells resulted in increased liver immunopathology in H2-Db deficient mice. Upon NK cell depletion anti-viral T cell immunity can be promoted to prevent severe symptoms during hepatic viral infection even during uniform MHC-I expression.

Discussion

The present study identified that uniform expression of MHC-I molecules can affect liver cell death, and severe CTL induced immunopathology following infection with LCMV. Mice lacking the MHC-I molecules H2-Db and to a lesser extent H2-Kb, exhibited enhanced CTL immunity. However, the enhanced $CD8^+$ T cell response did not result in a strikingly augmented clearance of the virus in the *H2-D1*$^{-/-}$ mice compared to WT mice during chronic LCMV infection. Instead, the hyper CTL cell immunity caused sustained liver pathology and severe disease. Ablation of CTL in *H2-D1* knockout mice prevented virus-induced liver pathology. Moreover, a further boost of anti-viral T cell immunity by NK cell depletion prevented pathology in absence of H2-Db. Collectively, our data provide evidence that during chronic infection, diverse MHC-I expression can prevent a hyper-responsive $CD8^+$ T cell immunity and protect the host from severe virus-induced immunopathology.

Expression of specific HLA molecules are associated with disease progression and outcome during HCV infections. Immunodominant HCV CTL epitopes of HLA-B27, HLA-B57, and HLA-B15 required several mutations to escape from CTL recognition. Therefore, HCV clearance was observed in most of the patients who express HLA-B*27, HLA-B*57, and HLA-B*15(19). On the contrary, HLA-A01* CTL epitopes only require one mutation to escape from CTL recognition. Consequently, HLA-A01 has not been identified as a protective HLA class I allele in HCV infection(19). In the LCMV setting both H2-Db and H2-Kb present the immunodominant epitope gp33 and gp34, respectively. Both H2-Db and H2-Kb can be considered as protective alleles in single MHC-I expression animals since enhanced CTL function was observed in *H2-D1*$^{-/-}$ *or H2-K1*$^{-/-}$ mice when compared to WT controls. However, we also observed slight but significant differences between H2-Db and H2-Kb expressing animals, suggesting that

expression of different MHC-I molecules can affect immunopathology and disease progression during viral infection. Notably, depletion of $CD8^+$ T cells abolished liver pathology, indicating that the functional CTL response in presence of a single MHC-I molecule expression setting triggered the pathology.

A narrow expression range of MHC-I molecules might influence immunopathology during viral infection. During infection with HIV homozygous expression of HLA molecules correlates with a rapid decline in $CD4^+$ T cells compared to patients with heterozygous HLA expression (17). Considering our data the rapid decline of $CD4^+$ T cell count in HLA homozygote individuals may be due to an increased anti-HIV CTL response targeting HIV-infected $CD4^+$ T cells (17). Our data showed that H2-D^b knockout mice exhibited a transient faster viral clearance during LCMV infection when compared to WT animals. In line with these data, no major significant viral clearance difference was observed from HCV or HBV infected patients between HLA class I heterozygote and HLA class I homozygote groups (15-17). Furthermore, establishment of liver fibrosis during infection with hepatitis C virus was not associated with homozygous expression of HLA –A, -B, or –C allels (18). Our data show that expression of a single MHC-I molecule is associated with increased liver immunopathology following infection with LCMV in comparison with expression of H2-D^b and H2-K^b. While overall our data support that heterozygote expression of MHC-I might be beneficial and prevents pathology during chronic infection, a detailed analysis of large patient cohorts and potentially overlapping effects need to be tested to validate these findings during human disease.Taken together we show that diverse expression of MHC-I molecules can prevent virus-induced, CTL mediated liver immunopathology during chronic LCMV infection.

Conflict of interest statement: The authors declare the following competing interests: H.C.X, P.P, R.W, K.S.L, and P.A.L declare that they are involved in the development of LCMV for clinical application in oncology in cooperation with or as advisors to Abalos Therapeutics GmbH.

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Author contribution:

H.C.X. initiated the study, performed experiments and wrote the paper. J.H., A.A.P., P.P., Z.Z., Y.Z., C.G.W.G, D.He performed experiments and wrote the paper. M.C, C.G.W.G., H.G., M.O, D.H, A.B, K.S.L. provided reagents, discussed the data, and wrote the paper. M.R. discussed the data and wrote the paper P.A.L. initiated the study, performed experiments and wrote the paper.

References:

1. Protzer U, Maini MK, Knolle PA. Living in the liver: hepatic infections. Nat Rev Immunol 2012;12:201-213.
2. Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay A. The interaction of hepatic lipid and glucose metabolism in liver diseases. J Hepatol 2012;56:952-964.
3. Reinke H, Asher G. Circadian Clock Control of Liver Metabolic Functions. Gastroenterology 2016;150:574-580.
4. Macpherson AJ, Heikenwalder M, Ganal-Vonarburg SC. The Liver at the Nexus of Host-Microbial Interactions. Cell Host Microbe 2016;20:561-571.
5. Rehermann B. Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells. Nat Med 2013;19:859-868.
6. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol 2005;5:215-229.
7. Knolle PA. Staying local-antigen presentation in the liver. Curr Opin Immunol 2016;40:36-42.
8. Huang LR, Wohlleber D, Reisinger F, Jenne CN, Cheng RL, Abdullah Z, Schildberg FA, et al. Intrahepatic myeloid-cell aggregates enable local proliferation of CD8(+) T cells and successful immunotherapy against chronic viral liver infection. Nat Immunol 2013;14:574-583.
9. Benseler V, Warren A, Vo M, Holz LE, Tay SS, Le Couteur DG, Breen E, et al. Hepatocyte entry leads to degradation of autoreactive CD8 T cells. Proc Natl Acad Sci U S A 2011;108:16735-16740.
10. Bottcher JP, Schanz O, Wohlleber D, Abdullah Z, Debey-Pascher S, Staratschek-Jox A, Hochst B, et al. Liver-primed memory T cells generated under noninflammatory conditions provide anti-infectious immunity. Cell Rep 2013;3:779-795.
11. Mueller SN, Ahmed R. High antigen levels are the cause of T cell exhaustion during chronic viral infection. Proc Natl Acad Sci U S A 2009;106:8623-8628.
12. Moskophidis D, Lechner F, Pircher H, Zinkernagel RM. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. Nature 1993;362:758-761.
13. McDevitt HO. Discovering the Role of the Major Histocompatibility Complex in the Immune Response. Annual Review of Immunology 2000;18:1-17.
14. Singh R, Kaul R, Kaul A, Khan K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. World J Gastroenterol 2007;13:1770-1787.
15. Thursz MR, Thomas HC, Greenwood BM, Hill AV. Heterozygote advantage for HLA class-II type in hepatitis B virus infection. Nat Genet 1997;17:11-12.
16. Hraber P, Kuiken C, Yusim K. Evidence for human leukocyte antigen heterozygote advantage against hepatitis C virus infection. Hepatology 2007;46:1713-1721.
17. Carrington M, Nelson GW, Martin MP, Kissner T, Vlahov D, Goedert JJ, Kaslow R, et al. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. Science 1999;283:1748-1752.
18. Patel K, Norris S, Lebeck L, Feng A, Clare M, Pianko S, Portmann B, et al. HLA class I allelic diversity and progression of fibrosis in patients with chronic hepatitis C. Hepatology 2006;43:241-249.

19. Nitschke K, Luxenburger H, Kiraithe MM, Thimme R, Neumann-Haefelin C. CD8+ T-Cell Responses in Hepatitis B and C: The (HLA-) A, B, and C of Hepatitis B and C. Dig Dis 2016;34:396-409.
20. Liang TJ, Block TM, McMahon BJ, Ghany MG, Urban S, Guo JT, Locarnini S, et al. Present and future therapies of hepatitis B: From discovery to cure. Hepatology 2015;62:1893-1908.
21. Baumert TF, Fauvelle C, Chen DY, Lauer GM. A prophylactic hepatitis C virus vaccine: a distant peak still worth climbing. J Hepatol 2014;61:S34-44.
22. Thimme R, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH, Chisari FV. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol 2003;77:68-76.
23. Lechner F, Wong DK, Dunbar PR, Chapman R, Chung RT, Dohrenwend P, Robbins G, et al. Analysis of successful immune responses in persons infected with hepatitis C virus. J Exp Med 2000;191:1499-1512.
24. Perarnau B, Saron MF, Reina San Martin B, Bervas N, Ong H, Soloski MJ, Smith AG, et al. Single H2Kb, H2Db and double H2KbDb knockout mice: peripheral CD8+ T cell repertoire and anti-lymphocytic choriomeningitis virus cytolytic responses. Eur J Immunol 1999;29:1243-1252.
25. Zinkernagel RM, Haenseler E, Leist T, Cerny A, Hengartner H, Althage A. T cell-mediated hepatitis in mice infected with lymphocytic choriomeningitis virus. Liver cell destruction by H-2 class I-restricted virus-specific cytotoxic T cells as a physiological correlate of the 51Cr-release assay? J Exp Med 1986;164:1075-1092.
26. Lang PA, Recher M, Haussinger D, Lang KS. Genes determining the course of virus persistence in the liver: lessons from murine infection with lymphocytic choriomeningitis virus. Cell Physiol Biochem 2010;26:263-272.
27. Masopust D, Murali-Krishna K, Ahmed R. Quantitating the magnitude of the lymphocytic choriomeningitis virus-specific CD8 T-cell response: it is even bigger than we thought. J Virol 2007;81:2002-2011.
28. Lang PA, Contaldo C, Georgiev P, El-Badry AM, Recher M, Kurrer M, Cervantes-Barragan L, et al. Aggravation of viral hepatitis by platelet-derived serotonin. Nat Med 2008;14:756-761.
29. Xu HC, Grusdat M, Pandyra AA, Polz R, Huang J, Sharma P, Deenen R, et al. Type I interferon protects antiviral CD8+ T cells from NK cell cytotoxicity. Immunity 2014;40:949-960.
30. Gautheron J, Gores GJ, Rodrigues CMP. Lytic cell death in metabolic liver disease. J Hepatol 2020;73:394-408.
31. Iwamoto N, Ito H, Ando K, Ishikawa T, Hara A, Taguchi A, Saito K, et al. Upregulation of indoleamine 2,3-dioxygenase in hepatocyte during acute hepatitis caused by hepatitis B virus-specific cytotoxic T lymphocytes in vivo. Liver Int 2009;29:277-283.
32. Rua R, Lee JY, Silva AB, Swafford IS, Maric D, Johnson KR, McGavern DB. Infection drives meningeal engraftment by inflammatory monocytes that impairs CNS immunity. Nat Immunol 2019;20:407-419.
33. Wohlleber D, Kashkar H, Gartner K, Frings MK, Odenthal M, Hegenbarth S, Borner C, et al. TNF-induced target cell killing by CTL activated through cross-presentation. Cell Rep 2012;2:478-487.
34. Guidotti LG, Inverso D, Sironi L, Di Lucia P, Fioravanti J, Ganzer L, Fiocchi A, et al. Immunosurveillance of the liver by intravascular effector CD8(+) T cells. Cell 2015;161:486-500.

35. Holst PJ, Orskov C, Qvortrup K, Christensen JP, Thomsen AR. CCR5 and CXCR3 are dispensable for liver infiltration, but CCR5 protects against virus-induced T-cell-mediated hepatic steatosis. J Virol 2007;81:10101-10112.
36. Lakhani SA, Masud A, Kuida K, Porter GA, Jr., Booth CJ, Mehal WZ, Inayat I, et al. Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. Science 2006;311:847-851.
37. Brenner D, Blaser H, Mak TW. Regulation of tumour necrosis factor signalling: live or let die. Nat Rev Immunol 2015;15:362-374.
38. Karki R, Sharma BR, Tuladhar S, Williams EP, Zalduondo L, Samir P, Zheng M, et al. COVID-19 cytokines and the hyperactive immune response: Synergism of TNF-alpha and IFN-gamma in triggering inflammation, tissue damage, and death. bioRxiv 2020.
39. Jordan MB, Hildeman D, Kappler J, Marrack P. An animal model of hemophagocytic lymphohistiocytosis (HLH): CD8+ T cells and interferon gamma are essential for the disorder. Blood 2004;104:735-743.
40. Lang PA, Lang KS, Xu HC, Grusdat M, Parish IA, Recher M, Elford AR, et al. Natural killer cell activation enhances immune pathology and promotes chronic infection by limiting CD8+ T-cell immunity. Proc Natl Acad Sci U S A 2012;109:1210-1215.
41. Waggoner SN, Cornberg M, Selin LK, Welsh RM. Natural killer cells act as rheostats modulating antiviral T cells. Nature 2011;481:394-398.
42. Cook KD, Whitmire JK. The depletion of NK cells prevents T cell exhaustion to efficiently control disseminating virus infection. J Immunol 2013;190:641-649.

Figure Legends

Figure 1: H2-Db expression protects animals from lethal LCMV induced liver pathology

(a) WT and *H2-D1*$^{-/-}$ mice were infected with 2x10^4 pfu of LCMV Docile. **(a)** Blood glucose concentration was monitored (starting with n=7 animals). **(b)** WT, *H2-D1*$^{-/-}$, and *H2-K1*$^{-/-}$ mice were infected with 2x10^4 pfu of LCMV-Docile and survival was monitored (n=6-7). **(c)** WT and *H2-D1*$^{-/-}$ mice were infected with 2x10^4 pfu of LCMV Docile. At day 9 post infection ALT, AST, and LDH activity in the serum of control and *H2-D1*$^{-/-}$ mice was determined (n=6). (Error bars show SEM, *p < 0.05, **p <0.01, and ***p < 0.001, between the indicated groups).

Figure 2: H2-Db is rapidly up-regulated in liver tissue following LCMV infection.

C57BL/6 mice (H2-b) were infected with 2x10^4 pfu of LCMV Docile. 4 days post infection, **(a)** *H2-D1* and *H2-K1* mRNA was quantified by RT-PCR using RNA isolated from liver tissue (n=6). **(b)** Beta-2 microglobulin protein expression in liver tissue was determined by immunoblot analysis (n=3). **(c)** Expression of H2-Db (upper panels) and H2-Kb (lower panels) was measured in different cell types of single cell suspended liver tissue from infected and naïve animals by flow cytometry (n=7 for Kupffer cells and hepatocytes, n=6 for endothelial cells, average of MFI is listed on the upper right corner). **(d)** Expression of H2-Db (upper panel) and H2-Kb (lower panel) was determined by flow cytometry in different immune cells in spleen tissue (n=7, average of MFI is listed on the upper right corner). (Error bars show SEM, *p < 0.05, **p <0.01 between the indicated groups).

Figure 3: H2-Db protects liver tissue during LCMV infection.

WT and *H2-D1*$^{-/-}$ mice were infected with 2x10^4 pfu of LCMV Docile. **(a-i)** At day 9 post infection, **(a)** Sections of snap frozen liver tissue were analyzed using H&E staining (one representative set of images of n=5 is shown, scale bar=50μm, dashed line indicates the lesion areas). Sections of snap frozen liver tissue were analyzed for **(b-c)** TUNEL **(d-e)** cleaved Caspase-3, and **(f-g)** cleaved Caspase-8 expression was determined and quantified using fluorescent staining of tissue sections (one representative set of images of n=8 is shown, scale bar=50 μm; three fields of each section were analyzed for the frequency of TUNEL, cleaved Caspase-3, or cleaved caspase-8 positive cells out of total Dapi positive cells, n=24). (Error bars show SEM, $^{***}p < 0.001$ between the indicated groups).

Figure 4: H2-Db knockout mice exhibited enhanced CTL immunity during LCMV infection.

(a) WT and *H2-D1*$^{-/-}$ mice were infected with 2x10^4 pfu of LCMV-Docile. Effector (KLRG1$^+$ IL-7R$^-$ CD62L$^-$), effector memory (KLRG1$^+$ IL-7R$^+$ CD62L$^-$, KLRG1$^-$ IL-7R$^+$ CD62L$^-$) and central memory (KLRG1$^-$ IL-7R$^+$ CD62L$^+$) markers were measured on tet-gp34$^+$ T cells in the blood at day 6 post infection (mean of n=13-14 is shown). **(b-g)** WT and *H2-D1*$^{-/-}$ mice were infected with 200 pfu of LCMV-Docile. **(b)** Tet-gp34$^+$ T cells were determined in the blood at the indicated time points (n=11). **(c)** Effector, effector memory, and central memory T cell subsets shown as a proportion of tet-gp34$^+$ T cells were determined in the blood of from WT and *H2-D1*$^{-/-}$ mice at day 12 post infection (mean of n=11 is shown). **(d)** Surface molecule expression was measured on tet-gp34$^+$ T cells as indicated (one representative set of n=11 is shown). **(e)** 12 days after infection, single cell suspended splenocytes were re-stimulated with LCMV-specific epitopes as indicated followed by staining for IFN-γ (n=8-9). **(f)** 8 days post infection, virus titers were determined in the spleen, liver, lung, and kidney tissues (n=6). (Error bars show

SEM, $^{*}p < 0.05$, $^{***}p < 0.001$ and ns indicates statistically not significant between the indicated groups).

Figure 5: CD8^{+} T cells trigger liver cell apoptosis in H2-Db infected knockout mice. CD8^{+} T cell –depleted, non-depleted WT and *H2-D1$^{-/-}$* mice were infected with 2x10^{4} pfu of LCMV-Docile. At day 9 post infection, sections of snap frozen liver tissue were analyzed for **(a+b)** cleaved Caspase-8 (n=7-9), **(c+d)** cleaved Caspase-3 (n=8), and **(e+f)** TUNEL (n=8). (One representative set of images is shown, scale bar = 50µm; for quantification, three fields of each section were analyzed for the frequency of cleaved Caspase-8 (n=21-27), cleaved Caspase-3 (n=24), or TUNEL (n=24) positive cells out of total Dapi positive cells). (**g**) Sections of snap frozen liver tissue were analyzed for H&E staining at day 9 post infection (one representative set of images of n=4 is shown, scale bar=50µm, dashed line indicates the lesion areas). **(h)** ALT, AST, and LDH activities in the serum at day 9 post infection were measured (n=7-9). (Error bars show SEM, $^{*}p<0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and ns indicates statistically not significant between the indicated groups).

Figure 6: CD8^{+} T cell depletion restores the survival of chronically infected H2-Db deficient mice. CD8^{+} T cell –depleted, non-depleted WT and *H2-D1$^{-/-}$* mice were infected with 2x10^{4} pfu of LCMV-Docile. **(a)** IFN-γ was measured in serum at the indicated time points post infection (n=3-8). **(b)** Blood glucose concentration was monitored over the course of infection (starting with n=6-7 animals). **(c)** Survival was monitored over time (n=7). **(d+e)** NK1.1^{+} cell–depleted, non-depleted WT and *H2-D1$^{-/-}$* mice were infected with 2x10^{4} pfu of LCMV-Docile. **(d)** tet-gp34^{+} T cells were determined at day 8 post infection (n=3-4).

(e) Survival was monitored (starting animals n=7 to 8). (Error bars show SEM, *p<0.05, **p < 0.01, ***p < 0.001 and ns indicates statistically not significant between the indicated groups).

Figure S1: H2-Db expression altered liver metabolism after LCMV infection.

(a) Serum ALT levels were quantified from naïve WT, *H2-D1*$^{-/-}$, *H2-K1*$^{-/-}$ mice. Serum from day 9 $2x10^4$ pfu LCMV Docile infected WT animals was used as positive control (n=4). **(b-d)** WT and *H2-D1*$^{-/-}$ mice were infected with $2x10^4$ pfu of LCMV Docile. Liver tissue metabolites from day 9 infected *H2-D1*$^{-/-}$ and WT animals were analyzed. Relative expression levels of **(b)** glycerophospholipids, **(c)** acylcarnitines, **(d)** aminoacids, **(e)** biogenic amines are shown (n = 5-6). (*p < 0.05, **p <0.01, and ***p < 0.001, and ns indicates statistically not significant between the indicated groups).

Figure S2: MHC-I is up-regulated mainly on Kupffer and endothelial cells in liver tissue during infection.

C57BL/6 mice were infected with $2x10^4$ LCMV Docile and liver **(a+b)** and spleen **(c+d)** tissue was harvested 4 days post infection. **(a)** Gating strategy of single cell suspended liver tissue is shown. **(b)** MFI of H2-Db (upper panel) and H2-Kb (lower panel) was measured in different cell subsets of single cell suspended liver tissue from naïve and infected animals by flow cytometry (n=7 for Kupffer cells and hepatocytes, n=6 for endothelial cells). **(c)** Gating strategy of immune cell subsets in single cell suspended splenocytes is illustrated. **(d)** MFI of H2-Db (upper panels) and H2-Kb (lower panels) was determined by flow cytometry on different immune cell subsets in splenocytes from naïve and infected animals (n=7). (Error bars show SEM, *p < 0.05, **p < 0.01, ***p < 0.001 and ns indicates statistically not significant between the indicated groups).

Figure S3: Virus distribution between WT and H2-Db deficient animals is similar during chronic viral infection.

WT and *H2-D1*$^{-/-}$ mice were infected with 2x10^4 pfu of LCMV-Docile. **(b)** LCMV nucleoprotein (NP) was visualized by immunohistochemistry of sections from liver tissue of WT and *H2-D1*$^{-/-}$ mice 9 days post infection (n=5-6, scale bar=100µm). **(c)** Virus titers were determined from spleen, liver, lung, and kidney tissue (n=8). (Error bars show SEM, ns indicates statistically not significant between the indicated groups).

Figure S4: H2-Db deficient animals exhibit enhanced liver cell apoptosis during chronic infection.

WT and *H2-D1*$^{-/-}$ mice were infected with 2x10^4 pfu of LCMV-Docile. At day 6 and 8 post infection, sections of snap frozen liver tissue were analyzed for TUNEL **(a+b)** and cleaved Caspase-3 **(c+d)** expression using immunofluorescence (one representative set of images of n=3 is shown, scale bar=50 µm. For quantification, three fields of each section were analyzed). **(e+f)** At day 9 post-infection, liver tissue Caspase-3 cleavage was determined using immunoblot analysis (n=5 for WT and *H2-D1*$^{-/-}$, n=3 for naive *H2-K1*$^{-/-}$). **(g+h)** At day 6 and day 8 post infection, sections of snap frozen liver tissue were analyzed for cleaved Caspase-8 expression using immunofluorescence (one representative set of images of n=3 is shown, scale bar=50µm. For quantification, three fields of each section were analyzed). **(i+j)** At day 9 post-infection, liver tissue Caspase-3 cleavage was determined using immunoblot analysis (n=5 for WT and *H2-D1*$^{-/-}$, n=3 for naive *H2-K1*$^{-/-}$). (Error bars show SEM, *p < 0.05, **p < 0.01, ***p < 0.001 and ns indicates statistically not significant between the indicated groups).

Figure S5: Increased effector T cells were observed in H2-Db deficient animals during viral infection.

(a-c) WT and *H2-D1*$^{-/-}$ mice were infected with 200 pfu of LCMV-Docile. **(a)** Gating strategy of T cell subsets is shown. **(b)** T cell subsets were determined on tet-gp34^{+} cells in the blood at day 8 or day 20 post infection (mean of n=11 is shown). **(c)** Standardized MFI of surface molecules on tet-gp34^{+} T cells was measured at indicated time points (n=11). **(d-f)** WT and *H2-K1*$^{-/-}$ mice were infected with 200 pfu of LCMV-Docile. At day 12 post infection **(d)** frequency of tet-gp33^{+} and tet-np396^{+} CD8^{+} were quantified in both blood and spleen tissue (n=7). **(e)** KLRG1^{+}IL-7R^{-}CD62L^{-} CD8^{+} T cell frequency of tet-gp33^{+} or tet-np396^{+} LCMV specific T cells was monitored in blood tissue (n=7). **(f)** WT and *H2-D1*$^{-/-}$ mice were infected with 200 pfu of LCMV-Docile. 12 days post infection, virus titers were determined in the spleen, liver, lung, and kidney tissue (n=5-6). (Error bars show SEM, *p < 0.05, **p < 0.01, ***p < 0.001 and ns indicates statistically not significant between the indicated groups).

Figure S6: Virus replication does not directly cause the immunopathology in H2-Db deficient animals.

CD8^{+} T cell–depleted, non-depleted WT and *H2-D1*$^{-/-}$ mice were infected with 2x10^{4} pfu of LCMV-Docile. Virus titers were determined from spleen, liver, lung, and kidney tissue at day 20 post infection (n=5-6).

Figure S7: TNF-α production was similar WT and H2-Db deficient mice following LCMV infection.

WT and *H2-D1*$^{-/-}$ mice were infected with 2x10^{4} pfu of LCMV-Docile. TNF-α was measured in the serum at the indicated time points post infection (n=3-4).

Figure S8: NK cell depletion recuses *H2-D1*$^{-/-}$ mice by enhancing CD8^{+} T cell immunity therefore faster clearance of viral antigens.

669 NK1.1^{+} cell–depleted, non-depleted WT and *H2-D1*$^{-/-}$ mice were infected with 2x10^{4} pfu
670 of LCMV-Docile. Virus titers were determined in the spleen, liver, lung, and kidney tissue
671 at day 20 post infection (n=6-8 for NK1.1^{+} cell–depleted, non-depleted WT and NK1.1^{+}
672 cell–depleted *H2-D1*$^{-/-}$ group, n=1 for NK1.1^{+} cell non-depleted *H2-D1*$^{-/-}$ group).